A Department of Ecology Report

Fecal Contamination Source Identification Methods in Surface Water

Abstract

This literature review discusses possible methods for differentiating between human and non-human sources of fecal contamination in surface waters. This document is written for the water quality investigator. Some of the more promising tools for assisting the investigator with source identification are described. The document is divided into sections on microbiological, chemical, and other methods for identifying sources of fecal contamination. A short description of each method is provided, along with some examples of studies that used the technique, and advantages and disadvantages of each method.

The study concludes that there is no easy, low-cost method for differentiating between human and non-human sources of bacterial contamination. Quantifying the contribution from different sources is as yet not possible. The most frequently used and well tested method at this time is genetic finger- printing. Promising methods on the horizon include techniques to amplify DNA using polymerase chain reaction (PCR), multiple antibiotic resistance, bacteriophages, and methods using a combination of indicators. The report recommends that the Washington State Department of Ecology do further research on streptococcal population profiles, and periodically update this publication.

Introduction

In recent years nonpoint pollution has surpassed point sources as the major source of fecal contamination to surface water. The water quality standards for surface waters of Washington State currently use fecal coliform bacteria as an indicator of fecal contamination. Fecal coliform bacteria are a sub-group of the total coliforms that grow mainly in the intestines of warm-blooded animals, including man.

To control nonpoint sources it is important to be able to identify the source of bacterial pollution so clean-up efforts can be effective. Bacterial indicators such as fecal coliform do not give us information on the specific source of pollution.

Purpose

The focus of this document is to describe some of the current methods for distinguishing between human and non-human bacterial sources. The document describes some of the more promising

by Debby Sargeant

Ecology Report #99-345
October 1999

tools for assisting the water quality investigator with source identification. This document is not intended to be a comprehensive, detailed literature review of current methods available.

Report Organization

This document is divided into sections on microbiological, chemical, and other methods for identifying sources of fecal contamination. The microbiological section covers bacterial and viral indicators found in the intestines of warm-blooded animals. Chemical indicators are natural byproducts of human metabolism or activity. A short description of each method is provided along with some examples of studies that used the technique, and pros and cons of each method.

The pros and cons may include field and laboratory complexity, cost, or important considerations for the method. Appendix A contains a discussion of bacterial indicators. This information may be useful background for the reader before reviewing the section entitled Microbiological Methods for Identifying Sources.

Microbiological Methods for Identifying Sources

Fecal Coliform to Fecal Streptococci Ratios

Description

The ratio of fecal coliform (FC) to fecal streptococci (FS) concentrations has been used to try to differentiate human from non-human sources of fecal contamination. A ratio of four or greater is considered human fecal contamination, a ratio of less than 0.7 suggests non-human sources (Edwards et. al., 1997). The value of this ratio has been questioned because of variable survival rates of fecal streptococcus group species. *Streptococcus bovis* and *S. equinus* die off rapidly once exposed to aquatic environments, whereas *S. faecalis* and *S. faecium* tend to survive longer. Disinfection of wastewater appears to have a significant effect on the ratio of these indicators, which may result in misleading conclusions regarding the source of contaminants. The ratio is also affected by the methods for enumerating fecal streptococci. For these reasons, Standard Methods (APHA, 1998) does not recommend FC:FS ratios as a method for differentiating between human and non-human sources of fecal contamination.

The major weakness of this approach is that unless the FC and FS die off at identical rates, the ratio will gradually change and thus will no longer reflect the original ratio in the fresh fecal material. Since it is not always possible to judge the age of pollution the problem of differential die-off makes the FC:FS ratio an unreliable method of determining the sources of pollution. However, Feachem (1974) suggested that differential die-off rates could be used to help differentiate the sources. Feachem noted that enterococci survive better than fecal coliform, which survive better than *S. bovis* and *S. equinus*. If a series of FC and FS concentrations are obtained through time, an improved estimate of the pollution sources could be obtained. A predominantly human source should exhibit an initially high (>4) ratio which should then fall, whereas a non-human source should exhibit an initially low ratio (0.7) which should subsequently rise (Table 1).

Table 1. Summary of fecal source related to FC:FS ratios (Feachem, 1974).

Initial FC\FS ratio	Change through time of FC\FS	Probable fecal Source
> 4	Rise	Uncertain
	Fall	Human
< 0.7	Rise	Non-human
	Fall	Uncertain

Coyne and Howell (1994) reported some success with the FC:FS ratio method. They found FC:FS ratios can suggest the probable source of fecal contamination, but it relies on considerable educated guesswork, and the conclusions drawn should not be considered absolute.

If ratios are used in an attempt to provide information on possible sources, the following guidelines are recommended (Geldreich and Kenner, 1969; Coyne and Howell, 1994):

1. The pH range of waters being tested should be between 4.0 and 9.0 because fecal coliform die off quicker than fecal streptococci in acid or alkaline water.
2. Sampling should occur within 24 hours after waste deposition. The faster die-off rate of fecal streptococci will alter the ratio as time from contamination increases.
3. Sample near the point of discharge or as close as possible to the pollution source. Pollution from several sources can alter the ratio and confuse the issue. The test is the most meaningful when samples are taken either at the point of discharge into the receiving stream or within a limited distance.
4. Ratios should not be used when fecal streptococcal counts are less than 100/100 mL. It becomes difficult to distinguish fecal streptococci in wastes from those that occur naturally in soil and water.
5. FC:FS ratios are of limited value in waters where regrowth can occur.
6. The mean FC:FS ratio for a site is largely meaningless because the range of ratios is so great. Evaluating the frequency with which FC: FS ratios fall within certain indicative values is a more accurate predictor of fecal contamination source.
7. A single sample has little diagnostic use. Numerous samples and a thorough knowledge of the watershed are necessary.

Pros

The FC:FS method is an inexpensive, moderately complicated laboratory procedure. Sample collection is routine. Manchester Environmental Laboratory (MEL) can perform these tests.

Cons

The results from this method are questionable. Better results may be achieved if the recommended guidelines are followed, and if the field investigator has information on land use and the pollutant loading mechanisms in the watershed.

Streptococcal Population Profiles

Description

Studies have shown that there are differences in the fecal streptococcus group species composition among various types of animals. Devriese et al. (1987) found different percentages of various fecal streptococci in the feces of poultry, cattle, and other animals.

In 1987 Rutkowski and Sjogren examined all members of the genus *Streptococcus*, including non-enteric groups, from sewage treatment facilities and animal feces. An improved medium allowed the isolation of 3,314 streptococcal strains representing 17 species. Thirteen of these species were assembled into groups based upon similarities in ecology or physiology. Comparison of the proportions of these groups with samples from various sources allowed human sources to be distinguished from other animal populations. They were able to quantify differences in the distribution of *Streptococcus* for non-human, human, and dairy sources.

Pros

Laboratory time and effort is moderate because of the numerous species to identify. Cost of the laboratory test is moderate. MEL can perform this test.

Cons

More testing is needed to check the validity of this approach. Also, while individuals tend to have a fairly stable fecal population from one sampling period to another, within a given species there can be a large variation in relative numbers of organisms present. In an area with numerous sources, profiling would not give information on the amount of contribution from each source.

Species-Specific Indicators

Description

There are a number of bacterial strains that are more specific to certain animal species. These indicators could be used to determine if fecal pollution from specific species is present. Comparisons of the quantity of organisms could indicate areas where specific pollution control measures are needed. Several of these indicators could also be used in concert to determine sources. The narrative below gives a summary of some of the more promising species-specific indicators. Table 2 summarizes characteristics of each method.

Streptococcus bovis. *Streptococcus bovis* was proposed as an indicator of animal fecal pollution in 1955 by Cooper and Ramadan (Kator and Rhodes, 1994). Since then few investigators have evaluated its use as an indicator of animal versus human pollution in freshwater, and no evaluation is known to have been done for marine and estuarine waters.

S. bovis has been associated with primarily ruminants but it has also been found in the feces of dogs, cats, horses, pigs, and various birds. Its occurrence in human feces is estimated at 1-15% (Kator and Rhodes, 1994). *S. bovis* has a lower survival rate than fecal coliform and *E. coli* in fresh water. Detection in surface waters may indicate recent pollution.

Table 2. Characteristics of species specific indicators.

Bacterial Species-Group	Source Identified	Survival Rate	Use in Saline Waters	Quantification of Source	Lab Costs
Streptococcus bovis	Non-human sources, though it does occur in low numbers in humans.	Low, represents recent fecal contamination	Has been tested in freshwater; no studies done in estuarine or marine water.	No	Low to moderate
Clostridium perfringens	Point source sewage pollution.	High, may not represent recent fecal contamination	Has been tested in fresh water but more information is needed on survivability in marine water.	No	Moderate, anaerobic laboratory procedures required.
Bifidobacteria longum, B. adolescentis	Human sources of point or nonpoint pollution.	Low, represents recent fecal contamination	Has been tested in fresh water but more information is needed on survivability in marine water.	No	High, analysis best with gene probe assays. More work needs to be done on lab methods.
Bacteroides fragilis group	Human sources of recent point or nonpoint pollution.	Low, represents recent fecal contamination	Has been tested in freshwater and marine water. Particulates in sample may present problems for estuarine sampling.	No	High, analysis best with gene probe assays. More work needs to be done on lab methods.
Rhodococcus coprophilus	Domestic grazing farm animals.	Moderate persistence in the environment	More testing is needed to determine suitability for marine water.	No	High, fairly complex test. There may be problems with lab method and enumeration procedure

In 1996 Kator and Rhodes measured the occurrence of *S. bovis* in a watershed where animal bacterial pollution sources from wildlife and domestic animals were considered the primary sources. They found *S. bovis* throughout the entire watershed, supporting animal pollution sources (Fletcher et al., 1999).

Clostridium perfringens. *Clostridium perfringens* is an obligate anaerobic enteric bacterium. Its spores are generally more tolerant to environmental effects than other traditional enteric indicators. *C. perfringens* has been suggested as an alternative bacterial indicator of fecal pollution because it is primarily associated with human wastes, and it is widely distributed in feces, sewage, and polluted waters.

A study by Fujioka and Shizumura (1985) found high numbers of *C. perfringens* in chlorinated sewage treatment plant effluents, but it was nearly undetectable in waters impacted by only nonpoint sources.

In surface water impacted by point (sewage treatment plant) and nonpoint sources (animal keeping operations), *C. perfringens* spores were detected in decreasing concentrations for more than 10 km from the sewage treatment plant (Sorensen et al., 1986). The study concluded that *C. perfringens* spores were a useful indicator of point source impacts, due to bacteria in streams that are also impacted by agricultural nonpoint sources.

C. perfringens spores may survive in the environment much longer than most pathogens. Thus they may not represent recent fecal contamination. Research is underway to determine occurrence of *C. perfringens* in ground water (USEPA, 1997a).

Bifidobacteria. *Bifidobacterium* are obligate anaerobic, non-spore forming bacteria that are a major component of the human intestine. There is the potential to differentiate between human and non-human fecal pollution based on specific strains of this bacterium, *B. longum* and *B. adolescentis*. In studies cited by Kator and Rhodes (1994*), B. longum and B. adolescentis* accounted for 74% of the bifidobacterial strains isolated from human feces.

In another study, investigators were unable to isolate the strains of bifidobacteria dominant in human feces. High densities of bifidobacteria were found in samples from raw sewage and septic tanks (Resnick and Levin, 1981).

Unfortunately, they do not survive long in the environment. *Bifidobacterium* and *E. coli* survived in stored river water up to 48 hours (McCorquodale, 1996). A temperature survivability study showed *B. adolescentis* persists at 6º C moderately better than *E. coli*, but at higher temperature *B. adolescentis* survivability was significantly worse (Kator and Rhodes, 1994).

Bacteroides fragilis group. The *Bacteroides* species are obligate anaerobic bacteria that comprise a majority of microorganisms in the human digestive tract. The term *Bacteroides fragilis* group is used to describe dominant *Bacteroides* found in the human intestine that includes *B. fragilis*, *B. thetaiotaomicron*, and *B. distasonis*. A study showed that higher levels of the *B. fragilis* group were found in human feces (67-78%) and lower levels in non-human feces (7-11%) and house pets (25%) (Kreader, 1995). *Bacteroides* are somewhat tolerant of oxygen and can survive a few days in the environment (Fields, 1999). Laboratory culture techniques are

difficult, but with the advent of gene probe assays, detection of bacterium in the *B. fragilis* group has been more successful.

Rhodococcus coprophilus. *Rhodococcus coprophilus* is an aerobic bacterium that has been proposed as an indicator of domestic farm animal fecal pollution. As a fecal indicator it is unique because it is associated with the feces of domestic grazing farm animals, but it is not considered an active component of the rumen microbiota (Kator and Rhodes, 1991). Studies have shown that *R. coprophilus* is absent in human feces but consistently found in feces of cattle, sheep, pigs, horses, donkeys, farm-raised poultry, and sporadically in dog and seagull feces. *R. coprophilus* can persist in vitro for 17 weeks in nonfiltered freshwater at 5°, 20°, and 30° C (Mara and Oragui, 1985).

In a study done in Zimbabwe, Mara and Oragui found *R. coprophilus* to be a reliable indicator of non-human fecal pollution, but further work was needed to improve the medium and methods to make this a practical and useful indicator.

Bacteriophages/Coliphages and Virus

Description

Bacteriophage. Bacteriophages or phages are viruses that infect bacteria. A given phage strain may be able to grow inside several strains of bacteria. Bacteriophages are present wherever coliform bacteria are present.

Coliphage. A coliphage is a virus that specifically infects and replicates in *Escherichia coli* bacteria.

Coliphage survival characteristics and inability to reproduce outside their host make them good fecal indicators (McCorquodale, 1996). Coliphages are commonly sorted into two groups: the somatic phage and the male-specific (or F-specific) phage.

Male-specific phages are not common in humans and other animals. They are common in sewage, suggesting they can multiply in a sewage system. The use of male-specific RNA coliphages (FRNA phage) has been proposed as a potential sewage pollution indicator. The FRNA phage may also be source specific. There are four major serological groups (I-IV) that have been identified. Kator and Rhodes (1994) cited studies that showed group I phages were detected in only domestic farm and feral zoo animals; FRNA phages from pigs belonged to groups I and II, and those from humans groups II and III. Phages from group III were exclusively human. Another study found groups I, II, and III in domestic sewage from treatment plants in Japan. However, group I phages were at low frequencies and could have been derived from animal sources.

While the FRNA phage does represent fecal contamination, this phage has a relatively low occurrence in the population of about 1-5%. Preliminary results from a study by Sobsy indicated 50% of septic tanks with sewage were contaminated with these viruses, presumably because they can persist by infecting *E. coli* in fresh sewage. Unfortunately, an unpublished EPA study found no male-specific phage in septic tanks (EPA, 1997a).
Currently EPA is investigating better analytical methods for the coliphage, especially the male-specific phage. The study will also examine the types of somatic and male-specific phage that are most closely associated with human fecal contamination.

Bacteroides fragilis phage. Unlike the coliphage, the *Bacteroides fragilis* phage is highly specific to its host. *Bacteroides fragilis* phages were detected exclusively in human feces and sewage. Phages lytic to the most efficient host strain examined, *B. fragilis* HSP40, were recovered only from environmental areas subjected to sewage, and were not detected in nonpolluted areas or those occupied exclusively by wild animals (Tartera and Jofre, 1987). This, along with the inability of *B. fragilis* phage to multiply in fresh water, seawater, or sediment habitats (Tartera et al., 1989), make this phage a promising indicator of human fecal pollution. While some coliphages can persist for extended periods (hundreds of days in natural nonfiltered seawater) not much is known about the survival rates *of B. fragilis* phages in the environment.

Pros

Male-specific coliphage may be used to determine the source of fecal contamination. They may be poor indicators of human contamination in nonpoint areas, but they may be a useful indicator of domestic farm animal contamination.

The *Bacteroides fragilis* phage specifically indicates human fecal contamination.

MEL can perform bacteriophage testing.

Cons

More research is needed on phages to determine if they can be used to differentiate between human and non-human fecal contamination. Research is needed on cost-effective laboratory methods, specificity of phage to host, and survival in estuarine and seawater environments.

FRNA phages are found in low occurrence in humans. Unless it can be shown that FRNA phages occur at reasonably high rates in septic leachate, these phages may be poor indicators of human contamination in nonpoint areas. Laboratory methods at this time are difficult and costly. Large sample volumes are necessary.

B. fragilis phage is not an appropriate indicator in seawater, due to low population densities. Large sample volumes are necessary.

Multiple Antibiotic Resistance

Description

Multiple antibiotic resistance (MAR) is a relatively new method for differentiating between human and non-human fecal sources. This approach is based on the fact that bacteria from wildlife species are generally lacking in antibiotic resistance, while strains from humans and domestic animals exhibit varying MAR. For this procedure either *Escherichia coli* or fecal streptococci from different animal species are analyzed to determine the resistance pattern for several different types and strengths of antibiotics.
Parveen et al. (1997) showed that in an area impacted by point and nonpoint sources, 82% of all samples taken were resistant to one or more antibiotics. In areas impacted by nonpoint sources the MAR Index was 50% lower. Results showed significantly higher MAR in urban versus pristine watersheds. While resistance patterns have been measured, it has been difficult to use

that information to identify the sources of fecal pollution. Discriminant function analysis is a variation of multivariate analysis of variance and can be used to classify individuals into groups on the basis of the values of several classification variables (Wiggins, 1996). In the study by Wiggins, 74% of the isolates were correctly classified into one of six possible sources (beef, chicken, dairy, human, turkey, or wild). A current study in Oregon that focuses on human and dairy sources has been able to detect and identify the source 86% of the time (Moore, 1998).

Pros

The MAR method for differentiating between fecal sources is promising. In a study being conducted in Oregon, investigators hope to be able to quantify contributions from each source (Moore, 1999).

Cons

This method is time intensive for the field and laboratory work. The laboratory procedure is complicated and costly. This is a relatively new method and more research is needed to validate the method. At this time MEL does not perform this test, but could in the future.

Microbial Source Tracking using DNA Ribotyping/Genetic Fingerprinting

Description

Genetic fingerprinting involves isolating pure cultures of *E. coli* (or other enteric pathogens) from both the receiving water and the suspected sources. DNA is isolated from these pure bacterial strains. The bacterial DNA is cut into fragments using a restrictive enzyme. The resulting fragments are separated by molecular weight using electrophoresis. Hybridization with a labeled DNA probe creates a chemiluminescent pattern of the fragments containing ribosomal RNA (rRNA) genetic information. The resulting fragments are then probed for rRNA from know *E.coli* strains. A camera creates an electronic image of the rRNA banding pattern. The resulting banding patterns from the possible sources are then compared to the banding patterns from the water samples. If matching banding patterns are found then they are known to be derived from the same strain of E. coli (Puget Sound Notes, 1993). There are several techniques developed for amplification of DNA; a common one is polymerase chain reaction (PCR). The use of PCR and gene probes to identify specific bacterium provides information on the sources of fecal contamination, but enumeration of pathogens is not possible.

Several source identification studies have been conducted in Washington using this method. The procedure involves isolating pure cultures of *E. coli* strains from both the suspected sources and the receiving environment. DNA isolates from sources are then compared to isolates found in the receiving environment. Unfortunately, only a portion of the strains isolated from the receiving water can be matched. For example, in the Soos Creek Study (Samadpour, 1995), 71% of the source matches belonged to 57 identified strains, leaving 29% unmatched. In the Pipers Creek study, 43% (76 of 176) of the receiving water isolates exhibited a ribotype match from the sample source isolates (Herrera Environmental Consultants Inc, 1992).

Pros

This is an excellent method for determining some of the sources of fecal contamination in a watershed.

Cons

Laboratory analysis is expensive. Quantification of the contribution from each source is not possible at this time. Only a portion of the receiving water isolates can be identified, leaving a significant percentage of unknown origin. Fieldwork is intensive because numerous receiving water samples are necessary and fresh fecal samples from all possible sources must be collected. Currently, MEL does not perform this test.

Chemical Methods for Identifying Sources

Chemical indicators are natural byproducts of human metabolism or activity. Specific chemicals can be used as tracers to indicate sources or routes of contamination.

Detergents/Optical Brighteners

Description

Fluorescent whitening agents or optical brighteners are chemicals that have a high affinity for cotton and, when exposed to UV light, emit a blue color. Optical brighteners are associated with laundry detergents, and their presence in surface and ground waters may indicate discharge of human waste from sewage or septic tanks. Optical brighteners are measured with a scanning fluorometer and results are expressed as fluorescent intensity. Fletcher et al. (1999) noted that optical brighteners have been used as indicators of septic tank or sewage discharge with varying results. Several studies concluded that optical brighteners might be useful for comparisons within a watershed, but not for comparisons between watersheds because of the high variability in natural background fluorescence.

Pros

This method may be a useful indicator of on-site septic system or gray water discharge. Laboratory time and costs are minimal.

Cons

Field work is time intensive. This method does not detect failing on-site systems. Other methods such as dye testing and charcoal packets are preferable for testing on-site systems. Dye testing and charcoal detect a higher percentage of failing systems (Hofstadt, 1999).

Caffeine

Description

Caffeine detection has been proposed as an indicator of human fecal pollution. McCorquodale (1996) reported that a study done by U. S. Geological Survey (USGS) on the Mississippi River found the highest concentrations near metropolitan areas. King County Metro has used caffeine to investigate water quality problems in combined sewer overflows (CSOs) in Duwamish River and Elliot Bay (Shuman, 1998). Caffeine was detected in close proximity to CSOs that had recently discharged or were currently discharging. It was found regularly in CSO effluent samples. Caffeine levels must be in high concentrations before they are detectable. A dilution of more than 200:1 makes it difficult to detect caffeine.

Pros

The laboratory test is inexpensive. MEL can perform this test.

Cons

This is not an effective method to detect human source pollution. Caffeine must be in very high concentrations and close to the source to be detected.

Coprostanol

Description

Coprostanol is a fecal sterol present in the feces of humans and other higher mammals. It is formed by the bacterial breakdown of cholesterol. Individuals vary as to the amount of coprostanol they excrete. Attributes that make coprostanol a good chemical indicator of human pollution are that it is the primary sterol in domestic wastes and it is unaffected by physical factors like temperature and salinity. There are varying reports of the persistence of coprostanol in the environment. One study showed it degrades after excretion, usually disappearing in 20 to 25 days. Persistence in the environment can be affected by microbial breakdown. When discharged into marine waters, coprostanol will settle out into nearby sediment; subsequently transport of the settled particulate matter may affect distributions, making correlations distant from pollution sources difficult. It has been used to monitor sewage and to detect fecal pollution in live-aboard marinas (McCorquodale, 1996; Fletcher et al., 1999).

Currently a study is being conducted in Florida to determine if fecal sterols such as coprostanol, epicoprostanol, cholestanol, and epicholestanol can be used to determine sources of fecal pollution. Initial data show that in fresh fecal samples, the ratio between two of the sterols, coprostanol and cholestanol, may serve as a marker for human fecal bacteria. The work indicates that the coprostanol:cholestanol concentration ratio is greater than 1.0 in human feces. A ratio of 1.0 or less would suggest a mixed or mainly non-human source (Broward County DNR, 1999).

Pros

Coprostanol may have limited usefulness as an indicator of near-source bacteria pollution from sewage sources. MEL can perform this test.

Cons

More study is needed on this method if it is to be used for nonpoint sources. The laboratory analysis is complicated and expensive. Large sample volumes may be needed. Particulates collected for the water sampling seem to fall out quickly in relation to distance from the source (Page, 1999).

Other Methods for Identifying Sources

Fluorescent Dye Tracing

Description

Fluorescent dye and charcoal packets are used to determine if on-site septic systems are functioning properly. Fluorescent dye is introduced into the on-site sewage system via the toilet and laundry sink. Charcoal packets are placed at any suspected discharge points or seepage areas in the vicinity of the septic system drainfield. The packets are retrieved one to two weeks from the time of placement and analyzed for the presence/absence of dye. All positive dye results are followed up by collecting a fecal coliform bacteria sample from the exact location of the positive charcoal packet.

Pros

Dye testing of on-site systems is the best method to determine if an on-site system is failing or bacterial pollution from human waste is occurring. The test is time intensive but thorough.

Cons

The major downfall of this method is the requirement for landowner cooperation to investigate all possible sources. This method involves an intensive field sampling procedure.

Land Use Based Site Selection

Description

Information on land use can be used to select monitoring sites that bracket potential bacterial sources. Bacterial monitoring sites can be placed upstream and downstream of the potential source. Statistical methods such as the paired t-test can be used to determine if there is a significant difference in bacterial levels between sites. Because of the variability inherent in bacterial testing numerous sampling events may be necessary. Monitoring should be targeted to the season or time when pollution is most likely to occur.

Pros

Identifies areas and possible sources of bacterial pollution. Laboratory expenses are minimal compared to some methods. MEL can perform this test.

Cons

This method may be time intensive and require numerous samples. It identifies the area where pollution is occurring, but not the specific source. For example, if a farm site with numerous animals is identified as the source of pollution, the possibilities for sources at the farm site could be on-site sewage failure or animal waste.

Summary

There is no easy, low-cost method for differentiating between human and non-human sources of fecal bacterial contamination. Quantifying the contribution from each source is still not possible. The best approach for an investigator at this time is to consider the land uses and sources under investigation, and tailor the method or methods to fit the situation.

At times, a combination of methods is appropriate for discerning sources. Some considerations in choosing a method or methods are:

- Type of sources (human, non-human, sewage, on-site, domestic, or feral animal);
- Pollutant loading mechanism and time frame;
- Sample medium (marine, freshwater, groundwater, sediments, fish or shellfish tissue); and
- Budget.

For example, if human fecal contamination is suspected one might test for presence of bacterial or phage strains more specific to humans.

The most frequently used and well-tested method at this time is genetic fingerprinting. Promising methods on the horizon include techniques using PCR, multiple antibiotic resistance, and bacteriophages, as well as methods using a combination of indicators.

Because of the recent focus on nonpoint pollution, there is great deal of research being done on possible methods to determine the sources of fecal bacterial pollution. As this document is being written, many promising studies examining alternative methods are being conducted. Ecology must stay current on upcoming methods, so the best information is available to assist investigators in fecal bacterial source identification.

Recommendations

- A periodic update of this publication should be conducted. The update should review recent studies and new methods that have been considered.

- Ecology should research streptococcus population profiles as a low-cost means to differentiate between human and non-human sources.

References

APHA *et al*, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. Clesceri, L.S., A.E. Greenberg, and A.D. Eaton (eds). American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D.C.

Broward County Department of Natural Resource Protection, 1999. *The Causes of Bacteria In Our Waterways.*. Broward County Department of Natural Resource Protection, Fort Lauderdale, FL. Internet address: http://www.co.broward.fl.us/dni02002.htm. Accessed May 4, 1999.

Coyne, M.S., J.M. Howell, 1994. *The Fecal Coliform/Fecal Streptococci Ratio (FC/FS) and Water Quality in the Bluegrass Region of Kentucky*. Soil and Science News & Views, Vol. 15, No. 9. Internet address: http://hermes.ecn.purdue.edu:8001/cgi/convwqtest?soil-15-9.ky.ascii, accessed May 10, 1999.

Determan, T., 1991. The Fecal Coliform Standard and Shellfish Sanitation. Shorelands and Coastal Zone Management Program, Washington State Department of Ecology, Olympia, WA.

Devriese, L.A., A. Van De Kerckhove, R. Kilpper-Balz, and K.H. Schleifer. 1987. *Characterization and identification of Enterococcus species isolated from the intestines of animals*. Int. J. Syst. Bacteriology. 37:257-259.

Edwards, E., M. Coyne, T. Daniel, P. Vendrell, J. Murdoch, and P. Moore, 1997. *Indicator Bacteria Concentrations of Two Northwest Arkansas Streams in Relation to Flow and Season*. American Society of Agricultural Engineers. Vol. 40(1):103-109.

Feachem, R., 1974. *An Improved Role For Faecal Coliform to faecal Streptococci Ratios in the Differentiation Between Human and Non-Human Pollution Sources.* Water Research, Vol. 9. pp. 689-690.

Fields, K., 1999. Personal communication March 11, 1999. Assistant Professor of Microbiology at Oregon State University, Corvallis, OR.

Fletcher, M., P. Verity, M. Frischer, K. Maruya, and G. Scott, 1999. *Microbial Indicators and Phytoplankton and Bacterial Communities as Evidence of Contamination Caused by Changing Land Use Patterns*. Baruch Institute, University of South Carolina. Internet address: http://inlet.geol.sc.edu/luces2/fletcher.html. Accessed April 26, 1999.

Fujioka, R.S. and Shizumura, L.K., 1985. *Clostridium perfringens: a reliable indicator of stream water quality*. Journal Water Pollution Control Federation. 57:986-992.

Geldreich, E. and B. Kenner, 1969. *Concepts of Fecal Streptococci in Stream Pollution.* Journal Water Pollution Control Federation, Vol. 41, No. 8, Part 2, pp. 336-352.

Herrera Environmental Consultants, Inc., 1992. Pipers Creek: Bacteriological Source Tracking Investigation. Herrera Environmental Consultants, Inc., Seattle, WA.

Hofstadt, L., 1999. Personal communication May 5, 1999. Thurston County Environmental Health, Olympia, WA.

Kator, H., and M.W. Rhodes, 1994. Microbial and chemical indicators. In: C. Hackney and M. Pierson (eds.) Environmental Indicators and Shellfish Safety. Chapman and Hall Publishers, New York and London pp. 30-91.

Kreader, C., 1995. *Design and Evaluation of Bacterioides DNA Probes for the Specific Detection of Human Fecal Pollution.* Applied Environmental Microbiology, Vol. 61, No. 4, pp. 1171-1179.

Mara, D., and J. Oragui, 1985. *Bacteriological methods for distinguishing between human and animal faecal pollution of water: results of fieldwork in Nigeria and Zimbabwe*. Bulletin of the World Health Organization, Vol. 63 (4): 773-783.

McCorquodale, D., and C. Burney, M. Row, 1996. Indicators for Determining the Sources and Extent of Fecal contamination in Coastal Waters: An Annotated Bibliography. Technical Report 96-06. Broward County Department of Natural Resource Protection, Fort Lauderdale, FL.

Moore, J., 1998. "Progress Report-Antibiotic Resistance Project" Memorandum to Robert Baumgartner (DEQ), dated August 13, 1998. Oregon State University, Corvalis, OR.

--------, J., 1999. Personal communication March 11, 1999. Department Head, Bioresource Engineering of Microbiology at Oregon State University, Corvallis, OR.

Page, R., 1999. Personal communication May 26, 1999. Broward County Department of Natural Resources, Fort Lauderdale, FL.

Parveen, S., R. L. Murphree, L. Edmiston, C. W. Kaspar, K. M. Portier, and M. Tamplin, 1997. *Association of multiple antibiotic resistance profiles with point and nonpoint sources of E. coli in Appalachicola Bay*. Applied Environmental Microbiology. Vol. 63: No. 7 pp. 2607-12.

Puget Sound Notes, 1993. Bacterial Source Tracking-Studies in an Urban Seattle Watershed. Puget Sound Water Quality Authority, Olympia, WA.

Resnick, I. and M. Levin, 1981. *Assessment of bifidobacteria as Indicators of Human Fecal Pollution.* Applied Environmental Microbiology, September 42(3):433-8.

Rutlowski, A.A., and R. E. Sjogren, 1987. *Streptococcal Population Profiles as Indicators of Water Quality*. Water, Air, Soil Pollution. 34:273-284.

Samadpour, M. and N. Chechowitz, 1995. Little Soos Creek Microbial Source Tracking. University of Washington Department of Environmental Health.

Shuman, R. Personal communication. September 1998. Seattle King County METRO, Seattle, WA.

Sorensen, D., S. Eberl, R. Dicksa, 1989. *Clostridium Perfringens as a Point Source Indicator in Non-Point Polluted Streams*. Water Resources, Vol. 23, No. 2, pp. 191-197.

Tartera, C. and J. Jofre, 1987. *Bacteriophages Active against Bacteroides fragilis in Sewage-Polluted Waters.* Applied and Environmental Microbiology, Vol. 53, No. 7, pp. 1632-37.

Tartera, C., F. Lucena, J. Jofre, 1989. *Human Origin of Bacteroides fragilis bacteriophages Present in the Environment*. Applied Environmental Microbiology, Vol. 10: 2696-701.

Todar, K., 1998. *The Normal Bacterial Flora of Animals.* University of Wisconsin Department of Bacteriology, Bacteriology 303 Main Page. Internet address: http://www.bact.wisc.edu/Bact303/Bact303normalflora, accessed May 10, 1999.

U. S. Environmental Protection Agency, 1997a. Movement toward making the Ground Water (disinfection) Rule a Reality: Summary of U.S. EPA Meeting on Analytical Methods for Monitoring Microbes in Ground Water September 18-19, 1997 (Updated March 8, 1999). Cincinnati, OH. Internet address: http://www.groundwatersystems.com/gwdr.html accessed May 27, 1999.

------, 1997b. Draft 10/29/97 *Microbial Monitoring under GWDR: Analytical Methods.* Office of Ground Water and Drinking Water. Internet address: http://www.epa.gov/OGWDW/standard/montbl2.html, accessed May 12, 1999.

Wiggins, B., 1996. *Discriminant Analysis of antibiotic Resistance Patterns in Fecal Streptococci, a Method To Differentiate Human and Animal Sources of Fecal Pollution in Natural Waters*. Applied and Environmental Microbiology, November 1996, pp. 3997-4002.

Contacts

Debby Sargeant Washington State Department of Ecology
Environmental Assessment Program
(360) 407-6684

For additional copies of this publication, please contact Ecology's Publications Distribution Office at (360) 407-7472 and refer to publication number 99-345.

The Department of Ecology is an equal opportunity agency and does not discriminate on the basis of race, creed, color, disability, age, religion, national origin, sec, marital status, disabled veteran's status, Vietnam Era veteran's status, or sexual orientation.

If you have special accommodation needs or require this document in alternative format, please contact Shirley Rollins at (360) 407-6696 (voice). Ecology's telecommunication device for the deaf (TDD) number at Ecology Headquarters is (360) 407-6006.

Appendix A

Microbiological Indicators

Bacterial Pollution Indicators versus Fecal Bacterial Source Indicators

Indicators are used when quantifying possible impacts to water from animal and sewage waste. They are usually used as a surrogate for more harmful pathogens. It is impossible to try to identify all the enteric pathogens present in the water. The cost is too great and the techniques have not been developed to test for all known pathogens.

There are three important requirements of an indicator. It should be native to the intestinal tract, enter the water with fecal discharge, and be found in the presence of other enteric pathogens. The indicator should normally survive longer than their disease-producing companions. They should be easy to isolate and identify (McCorquodale, 1996).

Washington State Bacterial Indicators

Total Coliform

Total coliform was originally used as an indicator of fecal contamination in surface water. Total coliform includes all the aerobic and facultative anaerobic, gram-negative, non-spore forming bacilli that, when incubated at 35° C, can ferment lactose and produce gas within 48 hours. This definition includes the genera *Escherichia, Citrobacter, Klebsiella*, and *Enterobacter*. Not all of these organisms inhabit the intestinal tract of warm-blooded animals exclusively.

Fecal Coliform

The fecal coliform group is a sub-group of total coliform that grow mainly in the intestines of warm-blooded animals. These organisms may be separated from the total coliform group by their ability to grow at elevated temperatures. The most common member of this group is *Escherichia coli* (an enteric bacteria), but also includes *Klebsiella*, *Enterobacter*, and *Serratia* which can also be found free-living on plants and in soils (Determan, 1991).

Fecal Streptococci and Enterococci Groups

Another group of bacteria less numerous in human feces than coliform is fecal streptococci. This group contains a number of species of the genus *Streptococcus*. Fecal streptococci indicate the presence of fecal contamination by warm-blooded animals. It is not known to multiply in the environment like fecal coliform. At one time *S. faecalis* and *S. faecium* were thought to be more human-specific than other *Streptococcus* species. Other species have been observed in human feces but less frequently. At the same time, *S. bovis, S. equinus*, and *S. avium* are not exclusive to animals, although they usually occur at higher densities in animal feces (APHA, 1998).

Enterococci are a sub-group of the fecal streptococcus group. This group consists of a number of species of *Streptococci*, *S. faecalis, S. faecium, S. gallinarum*, and *S. avium.* The enterococci

portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination in surface waters. Water quality guidelines based on enterococcal density have been proposed for recreational waters (APHA, 1998).

Other Bacterial Indicators

The intestines of warm-blooded animals host an incredible variety of bacteria. Most bacteria are a part of the normal intestinal floral. The type and quantity of bacteria species present can vary depending on animal species (Table 3.). Testing for specific bacterial species more common to certain animals can give information on possible bacterial sources. Some of the more species-specific microbiological indicators are described below. It is important to remember that bacteria flora can vary among the same species due to diet or location. Also, bacteria can colonize other animals if the environment is hospitable.

Table 3. Numbers of viable bacteria found in the feces of adult animals: logarithm of viable count per gram of feces* (Todar, 1998).

Animal	*Escherichia coli*	*Clostridium perfringens*	*Streptococci*	*Bacteroides*	*Lactobacilli*
Cattle	4.3	2.3	5.3	0	2.4
Sheep	6.5	4.3	6.1	0	3.9
Horses	4.1	0	6.8	0	7.0
Pigs	6.5	3.6	6.4	5.7	8.4
Chickens	6.6	2.4	7.5	0	8.5
Rabbits	2.7	0	4.3	8.6	0
Dogs	7.5	8.4	7.6	8.7	4.6
Cats	7.6	7.4	8.3	8.9	8.8
Mice	6.8	0	7.9	8.9	9.1
Humans	6.7	3.2	5.2	9.7	8.8

* Median values from 10 animals.
Modified from Rosebury, T., 1962. Microorganisms Indigenous to Man. McGraw-Hill, New York, NY.